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Intraneuronal accumulation of misfolded tau protein induces overexpression of Hsp27 in activated astrocytes

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ABSTRACT

Accumulation of misfolded forms of microtubule associated, neuronal protein tau causes neurofibrillary degeneration typical of Alzheimer's disease and other tauopathies. This process is accompanied by elevated cellular stress and concomitant deregulation of heat-shock proteins. We used a transgenic rat model of tauopathy to study involvement of heat shock protein 27 (Hsp27) in the process of neurofibrillary degeneration, its cell type specific expression and correlation with the amount of insoluble tau protein aggregates. The expression of Hsp27-mRNA is more than doubled and levels of Hsp27 protein tripled in aged transgenic animals with tau pathology. The data revealed a strong positive and highly significant correlation between Hsp27-mRNA and amount of sarkosyl insoluble tau. Interestingly, intracellular accumulation of insoluble misfolded tau protein in neurons was associated with overexpression of Hsp27 almost exclusively in reactive astrocytes, not in neurons. The topological dissociation of neuronally expressed pathological tau and the induction of astrocytic Hsp27, GFAP, and Vimentin along with up-regulation of microglia specific markers such as CD18, CD68 and C3 point to cooperation of astrocytes, microglia and neurons in response to intra-neuronal accumulation of insoluble tau. Our data suggest that over expression of Hsp27 represents a part of microglia-mediated astrocytic response mechanism in the process of neurofibrillary degeneration, which is not necessarily associated with neuroprotection and which in contrary may accelerate neurodegeneration in late stage of the disease. This phenomenon should be considered during development of disease modifying strategies for treatment of tauopathies and AD via regulation of activity of Hsp27.

1. Introduction

Sporadic and familial tauopathies represent a significant group of neurodegenerative conditions; among them Alzheimer's disease (AD) is the most common form of dementia [43]. Abnormally modified, misfolded neuronal protein tau is the main pathological factor inducing neurodegeneration in tauopathies. Its intracellular accumulation in the form of argyrophilic neurofibrillary lesions (neurofibrillary tangles (NFTs) and neuropil threads) is a defining feature of AD and several other neuropsychiatric disorders [9,18,52,53]. Misfolding of tau is the result of either mutations in tau gene [21,47] or aberrant posttranslational modifications of tau protein, resulting mainly from truncation [5,20,39,40,59] and/or abnormal phosphorylation [19,24]. Insoluble abnormal tau protein depositions in human brain start developing very early in life—during childhood and puberty. It can be found in lower brainstem first as a pre-tangle material in proximal axons and somatodendritic compartment of neurons in locus coeruleus, which later spreads into cortical areas and converts into argyrophilic lesions [7,8]. The fibrillary tau forms represent a critical pathogenic factor, as shown in cellular models [4,26,61], while NFTs were proposed to have a cyto-protective effect [10,41]. The pathologically modified tau protein, which appears prior to or during the transition from monomeric state to higher molecular oligomers is considered a critical toxic intermediate that should be therapeutically targeted [32].

The up-regulation of small molecular weight heat shock protein (Hsp27) has been detected in both neurons and astrocytes of different animal species under stress and in neurodegenerative conditions [2,14,17,22,23,27,33,57]. The elevated levels of Hsp27 in the cortex of brains from AD patients were revealed by immunohistochemical and biochemical studies. Hsp27-positive astrocytes were detected in close proximity to senile plaques and neurofibrillary tangles, and also various glial cells in different tauopathies were found Hsp27-positive [1,33,44,46,48,58]. Hsp27 was described as a direct interaction partner

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of phosphorylated tau in the brain from humans suffering of AD and in vitro it was found to facilitate degradation and dephosphorylation of abnormally phosphorylated tau [45]. Furthermore, overexpression of Hsp27 ameliorates joint toxic effects of mutant APP and PS1 proteins in a mouse model [54]. Small heat shock proteins, a part of protein quality control machinery, may serve as the first line of defense against stress-induced cell damage by maintaining proteins in a folding-competent state [25]. Probably as a result of these protective properties, Hsp27 has roles in promotion of neuron survival and neurite outgrowth, cell differentiation and inhibition of oxidative damage and apoptosis [3,42,56]. Mutations in the HSPB1 gene could lead to neurodegenerative disorders [34]. The molecular mechanisms underlying Hsp27 neuroprotection in specific types of brain cells remain under investigation, its role as neuro-protective factor in protein aggregate-associated neurodegeneration has not yet been fully elucidated and is controversial especially in chronic neurodegenerative diseases [6,49].

Here we investigated whether the pathological truncated tau protein induces expression of small heat shock protein Hsp27 in rat model of neurofibrillary degeneration. We have analyzed expression levels of Hsp27 in various stages of neurofibrillary pathology and cell type specific localization of Hsp27. Our data demonstrate that formation of insoluble truncated tau protein, not soluble tau, is associated with elevated levels of Hsp27 gene and protein expression. More importantly, we present clear cell type specific dissection of neurofibrillary pathology and Hsp27 expression. Surprisingly, Hsp27 is not induced in the neurons that bear aggregated misfolded tau proteins but almost exclusively in astrocytes of degenerated brain regions. The cell type specific expression pattern should be considered during development of disease modifying strategies for treatment of tauopathies and AD based on regulation of activity of heat shock proteins, specifically Hsp27.

2. Material and methods

2.1. Animals expressing human truncated tau protein

The transgenic rat strain expressing truncated human tau protein has been described elsewhere [31,60] Briefly, the transgene was prepared by ligation of a human cDNA encoding truncated tau protein (aa151-391,4R) into the mouse Thy-1 gene immediately downstream of the brain-enhancer sequence replacing thymus enhancer. Transgenic rats were generated by pronuclear injection of 1-day-old rat embryos. All rats were housed under standard laboratory conditions with free access to water and food, and were kept under diurnal lighting conditions (12 h light: dark cycles, with light starting at 7:00 am). The experiments were approved by the State Veterinary and Food Committee of Slovak Republic, and by Ethic Committee of Institute of Neuroimmunology, Slovak Academy of Sciences. The animals were anesthetized and sacrificed according to ethical guidelines to minimize pain and suffering of experimental animals. Efforts were made to minimize the number of animals utilized.

2.2. RNA extraction and quantitation by RT-qPCR

Brain samples were dissected from control and transgenic rats, immediately frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated by TRI Reagent extraction method according to manufacturer's protocol (Sigma-Aldrich, USA). Resulting RNA was briefly air-dried, dissolved in 100 μl of RNAse-free water (Qiagen, Germany). RNA samples were kept frozen at −80 °C. Integrity of isolated total RNA samples was determined by Agilent 2100 Bioanalyzer using a RNA 6000 Nano Labchip kit (Agilent Technologies, USA). For transcriptomic analysis we have used high quality RNA samples (RNA Integrity Number; RIN = 7.5–8.7).

Synthesis of the first strand was carried out using the High capacity cDNA reverse transcription kit (Applied Biosystems, USA) according to the manufacturer's recommendations. Levels of Hsp27, GFAP, Vim, C3, CD18 and CD68 mRNA were determined using qPCR with GAPDH as a standard. Following TaqMan primer assays (FAM for target genes and VIC for reference gene) were used for gene expression analysis: Hsp27, Rn00583001_g1; GFAP, Rn00566603_m1; Vim, Rn00579738_m1; C3, Rn00566466_m1; CD18, Rn01427948_m1; CD68, Rn01495634_g1; and reference gene GAPDH, Rn99999916_s1 (Applied Biosystems). Comparative ddCt analysis was performed in order to compare gene expression in transgenic and wild type animals. Results are expressed as a fold change of target gene mRNA level in transgenic animals compared to an arbitrarily selected individual from the non-transgenic control rat group.

2.3. Extraction of sarkosyl insoluble tau

Sarkosyl insoluble tau (SiT) was isolated from brain tissues of 6 month old rats. Frozen brain samples were homogenized in 10 volumes of ice-cold extraction buffer [SL buffer: 20 mM Tris, pH 7.4; 800 mM NaCl; 1 mM ethylenglycoltetraacetic acid (EGTA)]; 1 mM ethylenediaminetetraacetic acid (EDTA); 0.5% β-mercapthoethanol, 10% sucrose; 1 mM Na3VO4; 20 mM NaF; supplemented with the Complete without EDTA (Roche Diagnostics, USA) protease inhibitors using an OMNI TH tissue homogenizer (OMNI International, USA). After incubation on ice for 5 min the homogenates were cleared by centrifugation at 20,000 ×*g* for 20 min at 4 °C. The supernatants were collected and the total protein concentration was determined using the BioRad protein assay (BioRad, USA). This supernatant was designed 1S. Solid sarkosyl (N-lauroyl sarcosine, Na-salt, Sigma) was added to 1S supernatant to achieve 1% concentration and stirred for 1 h. This material was centrifuged at 100,000 ×*g* for 1.5 h at room temperature (RT). Supernatant was collected and pellets were gently rinsed with 1 ml of the SL buffer and centrifuged again at 100,000 ×*g* for 20 min at RT. The pellets were designated 2P. Samples for SDS-PAGE were prepared by dissolving 2P preparation in 1× SDS sample loading buffer in 1/50 volume of the 1S.

2.4. Glial primary cell culture and aggregation of tau

Mixed glial culture containing astrocytes and microglia was prepared from cerebral cortices of newborn Sprague Dawley rats (1 day old). Cells were cultured in DMEM containing 10% FCS and 2 mM L-glutamine for 7 days and treated for 24 h with 1 μM heparin-aggregated truncated tau protein, which was prepared according to previously published protocol [35].

2.5. Western blotting

The expression level of total human truncated tau in the transgenic rats was determined by Western blot analysis using protein extracts from experimental animals. Sarkosyl insoluble tau proteins in 2P preparation were analyzed on 10% SDS-PAGE gel and Western blot as described previously [31]. The membranes were probed with anti-tau primary antibody DC25 (pan-tau monoclonal antibody DC25 recognizing residues 347–354, Axon Neuroscience SE, Bratislava, Slovakia) [38,60], and other primary antibodies listed in Table 1. Incubation with goat anti-mouse HRP-conjugated secondary antibody (Dako, Denmark) was subsequently performed. Labeled secondary antibody was visualized with a chemiluminescence detection system (Pierce, USA), and the signal was recorded with a LAS3000 CCD imaging system (Fujifilm, Japan). Densitometric data analysis and relative quantification of Western blots were performed by AIDA Biopackage software (Raytest, Germany) as described [12]. The amount of Hsp27 protein was analyzed in the 1S preparation, and the relative level of anti-Hsp27 signal was normalized to the Gapdh (both antibodies purchased from Abcam, USA).

2.6. Tissue preparation

Preparation of samples was performed as described previously [50]. Rats were deeply anesthetized with ketamine–xylasine, perfused intracardially, their brains were removed and post-fixed overnight in 4% paraformaldehyde in phosphate-buffered saline, cryoprotected with 15% and 25% sucrose solutions (subsequently overnight), frozen in 2-methylbutane (30 s at −42 °C) and transferred to dry ice. Sagittal brain sections (40 μm thick) were cut on a Leica CM1850 cryomicrotome.

2.7. Immunohistochemistry

For stereological analysis, every 12-th section was included in the series (section sampling fraction = 1/12). Brain tissue samples were treated with 80% formic acid (30 s.) to retrieve the antigen accessibility. Tissue sections were incubated with primary antibodies anti-Hsp27 (Abcam, UK), anti-GFAP (Abcam, UK) or anti-NeuN antibody (Millipore, USA) overnight at 4 °C. Sections were immunostained using the standard avidin–biotin-peroxidase method (Vectastain ABC kit) with VIP as chromogen. Finally, sections were counterstained by methyl green (Vector, USA). All the antibodies used in this study are listed in Table 1.

2.8. Stereological quantification

For stereological quantification, male rats of transgenic line TG72 (n = 3) in the terminal stage of transgenic phenotype and age-matched non-transgenic littermates WT controls (n = 3) were used. Stereological quantification of the numbers of Hsp27-positive neurons and astrocytes, GFAP-positive astrocytes and NeuN-positive neurons was performed using an optical fractionator approach on a modified light microscope (Olympus BX51) equipped with a computer-based stereological system (StereoInvestigator; MicroBrightField, USA). The region of interest was selected at low magnification (objective 4× UPlanFI) and counting was performed at high magnification using an objective with high numerical aperture (60× oil immersion objective, Olympus, UPlanFI, NA = 1.25) and oil condenser (Olympus, UAAC, PlanApo, NA = 1.40). Parameters of the stereological analysis (dissector base, sampling grid) were chosen according to density of the particular structure in the tissue — sparse Hsp27-positive cells required a denser grid than frequent GFAP-positive astrocytes, to ensure precise analysis with appropriately low coefficient of error. The parameters of stereological analysis are described in table 2.

2.9. Immunofluorescence

Co-localization experiments were performed on free-floating sections using the standard immunofluorescent staining procedure. After 20 min in 1% sodium borohydrate and 1 h blocking in 5% bovine serum albumin (Sigma), sections were incubated with primary anti-GFAP (1:500), AT8 (1:1000) and anti-Hsp27 (1:500) antibodies overnight at 4 °C. After washing, the sections were incubated for 1 h in secondary antibodies (1:1000) conjugated either with Alexa Fluor 546 or Alexa Fluor 488 fluorescent dyes (Invitrogen, Eugene, Oregon, USA). After washing, the sections were mounted onto slides using Vectashield mounting medium (Vector laboratories, USA) and examined with laser scanning confocal microscope LSM 710 (Zeiss).

2.10. Statistical analysis

Graphpad Prism (version 4.03 for Windows; Graph Pad Software, San Diego, CA, USA) was used to carry out the statistical analysis. Differences were analyzed with unpaired Student's *t*-test. Correlation analyses were performed adopting non-parametric Spearman correlation. Results were considered to be statistically significant if $p < 0.05$.

3. Results

3.1. Hsp27 is significantly upregulated in aged rats expressing truncated tau protein

Transgenic rat strain (Tg line #72) used in this study has been described elsewhere [31,60]. Expression of truncated tau protein in Tg line #72 leads to development of AD-like tau pathology in neurons of brainstem and spinal cord. To study the Hsp27 expression in the brain area directly affected by neurodegeneration, we performed all experiments on the whole brainstem tissue, which includes midbrain, pons and medulla oblongata. Firstly, we determined the developmental profile of Hsp27 in transgenic (TG) animals and their wild type (WT) littermates on Western blot (Fig. 1a). Very low amount of Hsp27 was found in early postnatal period (12 days) in both strains. The levels of Hsp27 changed in adulthood, exhibiting an age-dependent gradual increase with no apparent difference between TG animals and WT controls up to the 4th month of age. However, in the age of 6 months when pathological changes start to appear in transgenic population [31], the level of Hsp27 in TG animals suddenly increased to 2.8-fold of control animals value (Fig. 1b). To confirm these data, we repeated the experiment on larger group of animals. We have analyzed Hsp27 protein and mRNA level focusing on two developmental stages (4 and 6 months). We found slightly elevated Hsp27 at the 4th month of age in TG animals, whereas later, at the 6th month of age, we observed a 3-fold increase of Hsp27 levels compared to WT (Fig. 1c, d). The Hsp27 mRNA was slightly elevated in TG animals already at the age of 4 months (nearly 20% increase in comparison to WT). By the 6th month of age the expression of Hsp27 mRNA in TG animals has increased dramatically and was found about 2.3 times higher in comparison to WT animals of the same age (Fig. 1e). Interestingly, the level of soluble transgenic truncated tau protein remained stable in the adulthood of TG animals, whereas the amount of insoluble tau protein forms gradually increased as TG animals got older (months 2–6, Fig. 1f, g). In conclusion, Hsp27 mRNA and protein are significantly overexpressed in the brainstem of TG animals and this upregulation occurs concurrently with the increase of insoluble tau, independently on the pool of soluble transgenic tau protein. These data indicate that Hsp27 is induced by insoluble misfolded tau proteins and not by the soluble, although abnormal, tau.

Table 1
List of primary antibodies used in the study.

Antibodies	Species	Specificity	Dilution	Source
AT8	Mouse	Phosphorylated tau protein (Ser-202, Thr-205)	1:1000	Thermo Scientific (Cat. MN1020)
DC 25	Mouse	Tau protein, aa347–353	1:1000	Axon Neuroscience
GAPDH	Mouse	Glyceraldehyde-3-phosphate dehydrogenase	1:2000	Abcam (Cat. 9484)
GFAP	Mouse	Glial fibrillary acidic protein	1:500	Abcam (Cat. ab10062)
Hsp27	Rabbit	Heat shock protein 27	1:500	Abcam (Cat. ab12351)
NeuN	Mouse	Neuron-specific protein	1:500	Millipore (Cat. MAB377)

Table 2
Parameters of the stereological analysis.

	Dissector		Sampling						
	Base	Height	Grid	UGZ	BA	t			
Structure quantified (antibody)	(μm × μm)	(μm)	(μm × μm)	(μm)	(μm)	(μm)	∑OD	∑Q-	CE
Astrocytes (Hsp27)	80 × 80	15	500 × 500	2	40	22.2	2009	1144	0.085
Neurons (Hsp27)	80 × 80	15	500 × 500	2	40	22.2	2009	54	0.174
Astrocytes (GFAP)	70 × 70	15	900 × 900	2	40	21.2	629	1348	0.026
Neurons (NeuN)	70 × 70	15	600 × 600	2	40	20.8	1431	3169	0.034

3.2. Induction of Hsp27 is directly proportional to the amount of insoluble aggregated tau protein in transgenic animals

Development of insoluble tau protein, designed as sarkosyl-detergent insoluble pool of tau and its accumulation in affected brain areas is a prominent sign of neurofibrillary degeneration in human tauopathies. Similarly, in the rat Tg line #72 the load of sarkosyl insoluble tau protein (SIT) reflects the stage of neurofibrillary pathology and correlates with the life span of the TG animals [31]. To evaluate the relationship of Hsp27 expression and the stage of neurofibrillary pathology we have quantified the Hsp27 mRNA and SIT by RT-qPCR and Western blot, respectively, in the brainstem samples from a cohort of six-months old TG animals ($n = 20$, Fig. 2). The amount of Hsp27 mRNA in TG animals was 1.35 to 3.8 higher than the amount of mRNA detected in WT animals of the same age (compare with Fig. 1e), and the profile of Hsp27 mRNA overexpression in TG animals mirrored the highly variable profile of SIT in the contralateral brain region (Fig. 2a). Moreover, the Hsp27 mRNA fold increase strongly correlated with the amount of SIT, indicating an interplay between the expression of Hsp27 and the degree of neurofibrillary pathology in transgenic animals (Fig. 2c; $p = 0.0004$; $r = 0.72$).

3.3. Hsp27 is predominantly overexpressed in reactive astrocytes located in regions with tau pathology, rarely in neurons

Immunohistochemical staining revealed that in control rat brains, where no tau pathology was observed (Fig. 3a), Hsp27 was almost not present in astrocytes (Fig.3b) expressing GFAP protein (Fig.3c). On the contrary, in transgenic rat brain, where extensive neurofibrillary degeneration was found in the reticular formation of the brainstem (Fig. 3d), we detected a high number of reactive astrocytes expressing Hsp27 (Fig. 3e) and GFAP (Fig. 3f). Stereological quantification demonstrated significant increase of the number of GFAP-positive (reactive) astrocytes in TG brains compared to WT animals (1.7-fold increase; $p < 0.002$; Fig. 3g). Further, we observed a selective increase of Hsp27-positive reactive astrocytes in transgenic rat brains (16-fold increase; $p < 0.01$; Fig. 3h). Interestingly, more than 30% of the GFAP-positive astrocytes were also immunoreactive to Hsp27 in transgenic rat brain,

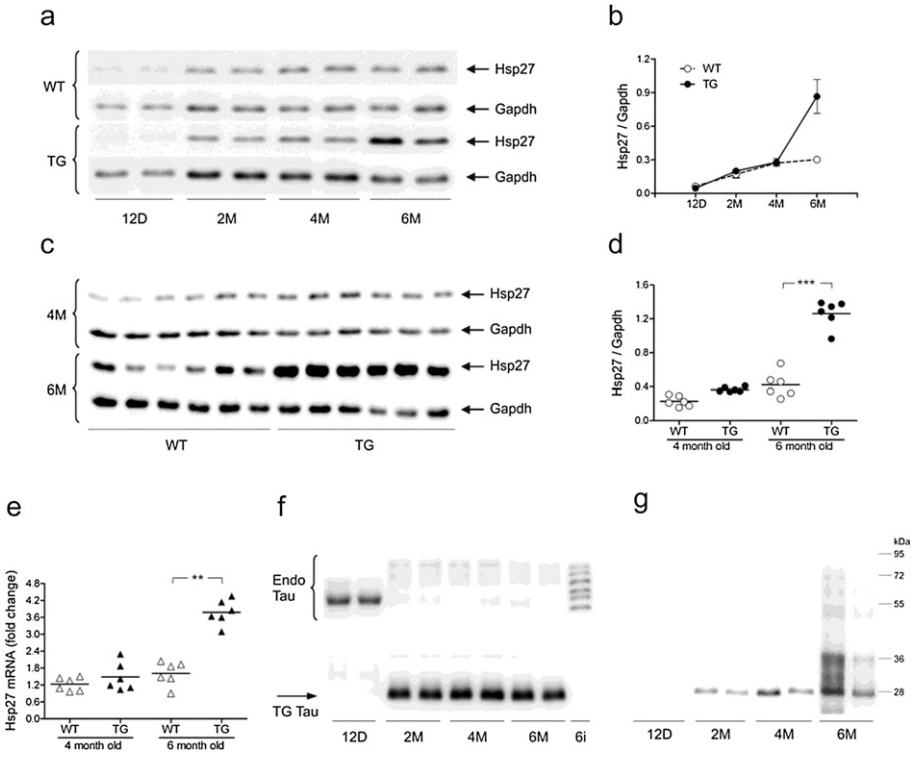

Fig. 1. Protein and mRNA levels of Hsp27 are significantly upregulated in aged rats expressing truncated tau protein. Western blot analysis of brainstem protein extracts revealed the profile of Hsp27 expression during development (a, b). Detailed comparison of WT and TG animals at 4th and 6th months of age showed significant increase of Hsp27 protein level in 6th month old TG animals (c, d). Expression of Hsp27 protein was normalized to Gapdh level ($n = 6$, $^{***}P < 0.001$). The upregulation of Hsp27 at the protein level was confirmed by mRNA expression analysis (e), $^{**}P = 0.002$. Soluble tau protein expression in TG animals was analyzed by pan-tau antibody (DC25) recognizing rat endogenous and human truncated tau forms (f). Recombinant tau protein isoforms (6i) were used as a control. Compared to the stable expression of soluble tau proteins the sarkosyl insoluble tau fraction increased with the age of TG animals (g) as determined by DC25.

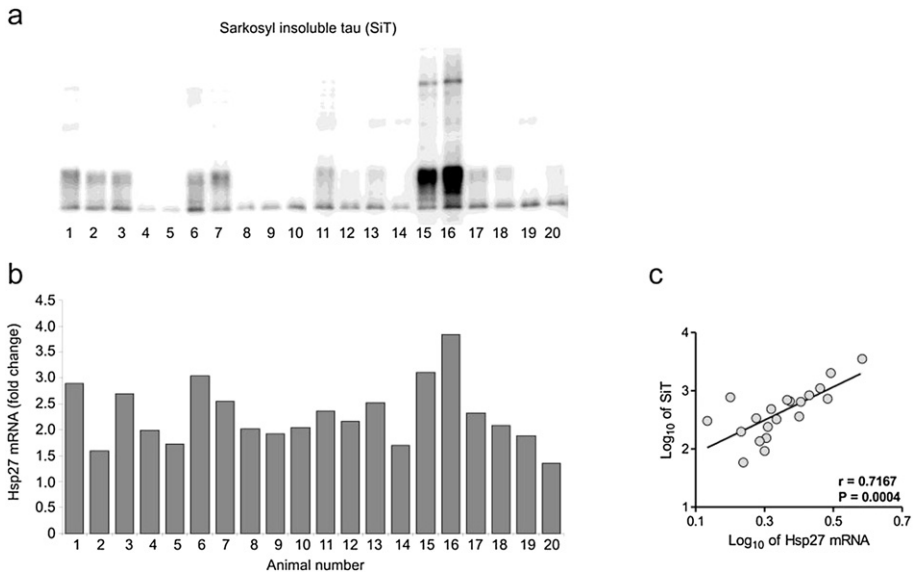

Fig. 2. Hsp27 mRNA correlates with insoluble, aggregated tau protein level. Western blot analysis using the pan tau antibody DC 25 revealed different amounts of insoluble and aggregated tau protein in brainstem of experimental animals (a). The Hsp27 mRNA fold change in TG animals (b) corresponds to the amount of the insoluble tau. We found statistically significant correlation between expression of Hsp27 mRNA and amount of sarkosyl insoluble tau (***P = 0.0004, r = 0.7167, n = 20).

whereas in control animals only 3% of astrocytes were immunoreactive to both markers ($p < 0.05$) (Fig. 3i). Confocal study supported our immunohistochemical data showing that significant part of GFAP positive astrocytes displayed increased expression of Hsp27 in TG animals (Fig. 4a–c). Moreover, we found that Hsp27 positive reactive astroglial cells were localized in a close proximity of AT-8 positive neurofibrillary

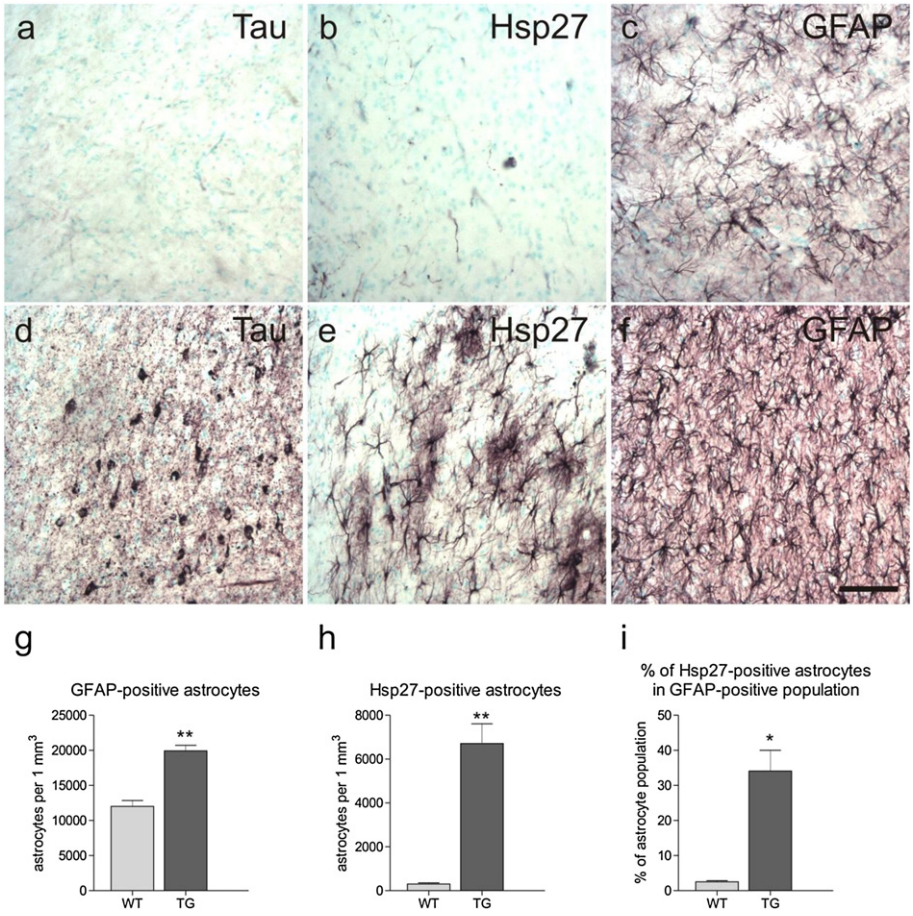

Fig. 3. Regional distribution of Hsp27 positive astroglia is parallel that of neurofibrillary tangles in transgenic rat brainstem. In the brainstem of control rat we did not observe any AT8 positive tau pathological lesions (a). Hsp27 positive astrocytes were present occasionally (b). Panel c shows GFAP positive astrocytes in control non transgenic rats. In contrast, in transgenic rat brain we found large number of AT8 positive neurofibrillary tangles and neuropil threads, mainly in the nuclei of reticular formation (d). In the area affected by neurofibrillary pathology, numerous Hsp27 and GFAP positive astrocytes were found (e, f). Stereological quantification showed increased number of GFAP and Hsp27 positive astrocytes in the brainstem of transgenic rats (g, h). We found that on average every 3rd GFAP positive astrocyte expressed also Hsp27 protein (i). Scale bar: 100 μm.

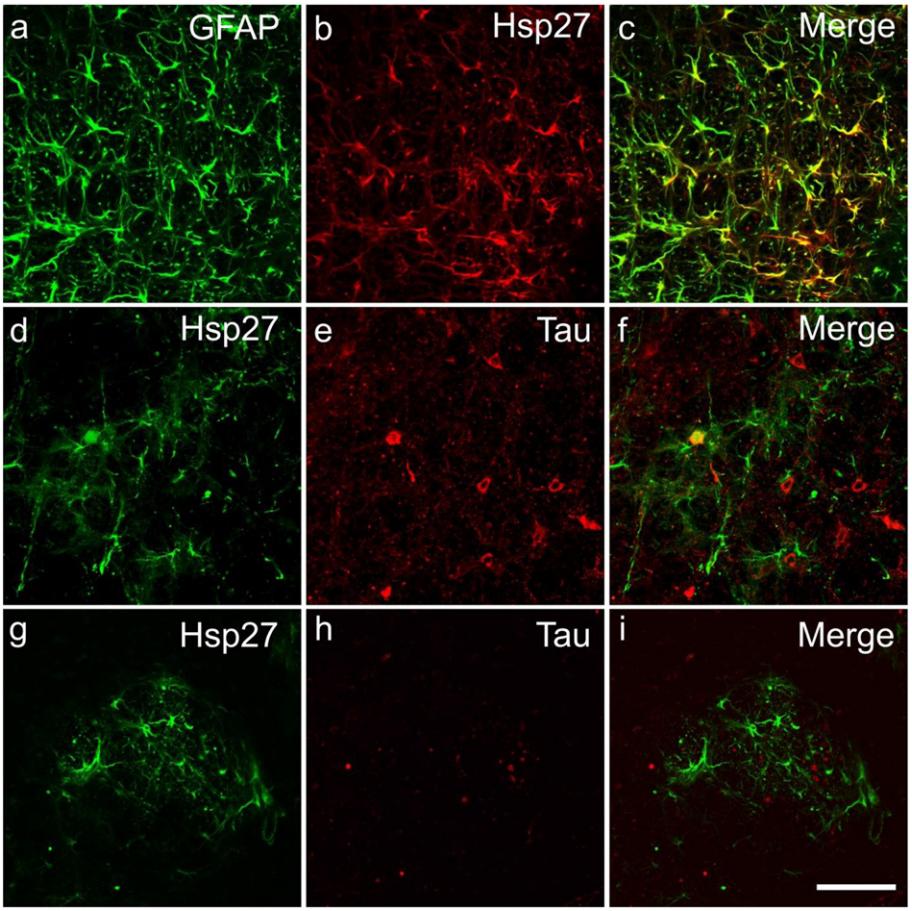

Fig. 4. Confocal microscopy showed that Hsp27 positive astrocytes are distributed in a close proximity of tau neurofibrillary lesions. A subgroup of GFAP positive astrocytes expressed high levels of Hsp27 (a–c). Hsp27 positive astrocytes were localized close to the AT8-positive tau neurofibrillary tangles (d–f) and dystrophic neurites (g–i). However, intraneuronal co-localization of Hsp27 and AT8 signals was very rare (f). Scale bar: 100 μm.

tangles (Fig. 4d–f) and AT8 positive dystrophic neurons (Fig. 4g–i); the co-localization of Hsp27 signal with degenerating neurons was very rare (Fig. 4f).

3.4. Tau neurodegeneration did not reduce the number of Hsp27 neurons in the brainstem of transgenic rats

In order to identify the impact of tau neurodegeneration on total neuronal numbers we stained both transgenic and control rat brains with neuron specific marker Neu-N (Fig. 5a,b). We found no difference in total number of NeuN-positive neurons when comparing TG and WT animals (Fig. 5e). It is known, that Hsp 27 is constrictively expressed in several sensory and motor neurons in the brainstem and spinal cord. In both control and transgenic rats, we have found extensive Hsp27 immunoreactivity in following brainstem nuclei: nucleus ambiguous, trigeminal motor nucleus, mesencephalic nucleus of the trigeminal nerve and facial nucleus (Fig. 5c, d). Interestingly, these types of neurons usually do not develop neurofibrillary pathology and no activated Hsp27 immunopositive astrocytes were present in close proximity of these neurons. We found a non-significant increase of Hsp27-positive neurons in TG rats (Fig. 5f). Overall, only 0.9% and 1.1% of all neurons were Hsp27-positive in WT and TG animals, respectively (Fig. 5g).

3.5. Aggregated tau induces Hsp27 expression in astroglial cell cultures

To provide further evidence for an induction of Hsp27 expression in astrocytes by aberrant tau protein, we exposed primary culture of astrocytes from WT animals to the in vitro aggregated human truncated tau protein prepared by incubation of recombinant tau with heparin [35]. As shown on Fig. 6a, heparin-aggregated truncated tau induced Hsp27 and GFAP proteins expression after short term (24 h) incubation. The increase in the level of both proteins was statistically significant (Fig. 6b, c). This experimental design mimics the influence of extracellular tau (released from neurons accumulating pathological tau) on astrocytes, and suggests that extracellular forms of tau may induce the expression of Hsp27 in astrocytes observed in TG animals.

3.6. The level of sarkosyl insoluble tau correlates with increased expression of astroglial and microglial markers in transgenic rat brain

To further characterize the relation of SIT and glial cells activation, we performed transcriptomic analysis of selected glial cell specific markers and key proteins of immune system in the brainstem tissue samples. We found highly significant positive correlations between the amount of SIT and mRNA levels of reactive astrocytes markers such as GFAP and Vimentin (Fig. 7a), as well as the markers of activated microglia, complement C3, surface antigens CD18 and CD68 (Fig. 7b). As expected, we observed strong and statistically significant correlations also between the mRNA levels of Hsp27 and all five examined markers (GFAP, Vimentin, C3, CD18 and CD68; Supplement Fig. 1). Our data suggest that it is astrocytes and microglia that respond to the intraneuronal accumulation of misfolded tau proteins via induction of an immune-stress response pathway including Hsp27.

4. Discussion

Posttranslational modifications of tau protein, such as pathological hyperphosphorylation and proteolytical truncation, lead to

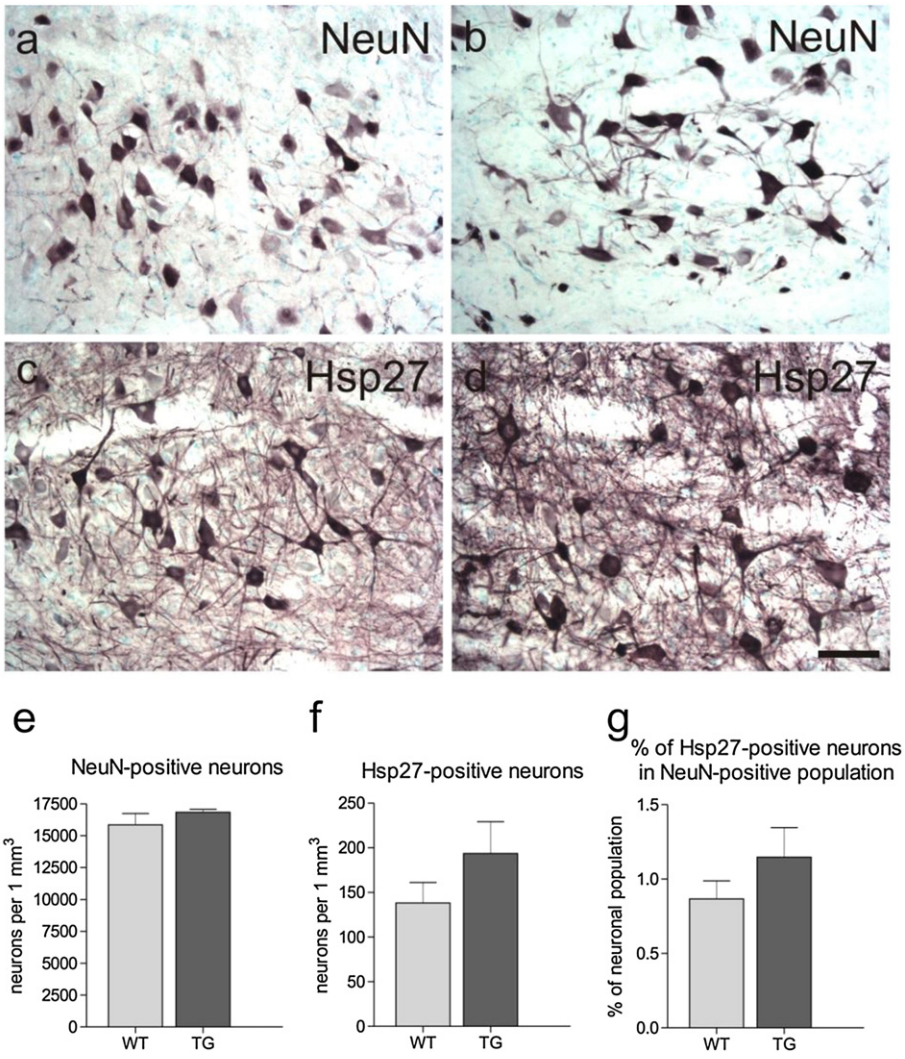

Fig. 5. Neurofibrillary pathology did not reduce the number of Hsp27 positive neurons in transgenic rat brain. Neuronal cells were labeled by neuronal marker Neu-N in both control and transgenic rat brain (a, b). The Hsp27 antibody recognized the same motor and sensory neuronal nuclei in control and transgenic rat brains (c,d). Stereological quantification of Neu-N positive brainstem neurons revealed the same neuronal number in control and transgenic rat brainstem (e). Similarly, the number of Hsp27 positive neurons in the brainstem was almost identical in control and transgenic rats (f). Number of Hsp27 positive Neu-N neurons was very low (about 1% of total neuronal population) and similar in control and transgenic brainstem (g). Figures (a–d) show Neu-N and Hsp27 staining of the facial nucleus in the rat brainstem. Scale bar: 50 μm.

neurodegeneration [19,40]. Truncation of tau protein participates in the early events in AD and occurs prior to formation of neurofibrillary tangles [5,37,40]. Recently, we and others showed that expression of the truncated tau protein induces neurodegeneration to a different extent in rodent experimental models [16,31,36,51,60]. Transgenic rats expressing human truncated tau develop neurofibrillary degeneration, similar to that observed in human tauopathy and AD [16,31,60], progressive decline of sensorimotor functions and impairment of several reflexes [28] and elevated oxidative stress [11]. These findings are interesting particularly when compared to recently described transgenic rats expressing mutated tau protein (P301L), which do not display any obvious neurofibrillary pathology, neuronal loss or glial activation [29].

An age dependent increase in the amount of sarkosyl-insoluble tau and the number of neurofibrillary tangles are the typical features of

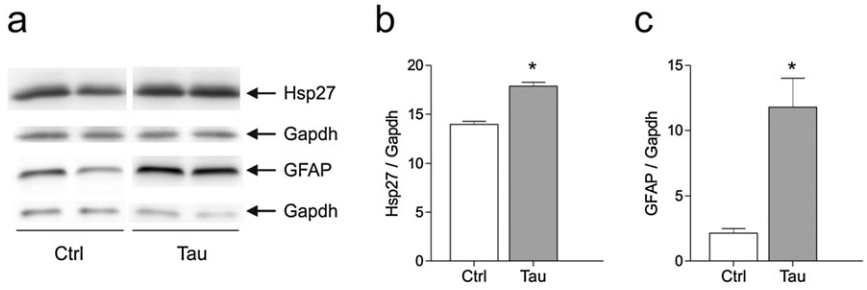

Fig. 6. Aggregated tau protein induces Hsp27 expression in astroglial cells in vitro. Incubation of heparin-aggregated truncated tau protein with astroglial cells for 24 h led to significant up-regulation of Hsp27 and GFAP protein levels when compared to control cells (a). Expression of Hsp27 and GFAP protein was normalized to Gapdh (b, c) revealing 1.3-fold increase of Hsp27 (*P = 0.0166) and 5.5-fold increase of GFAP (*P = 0.0127), respectively.

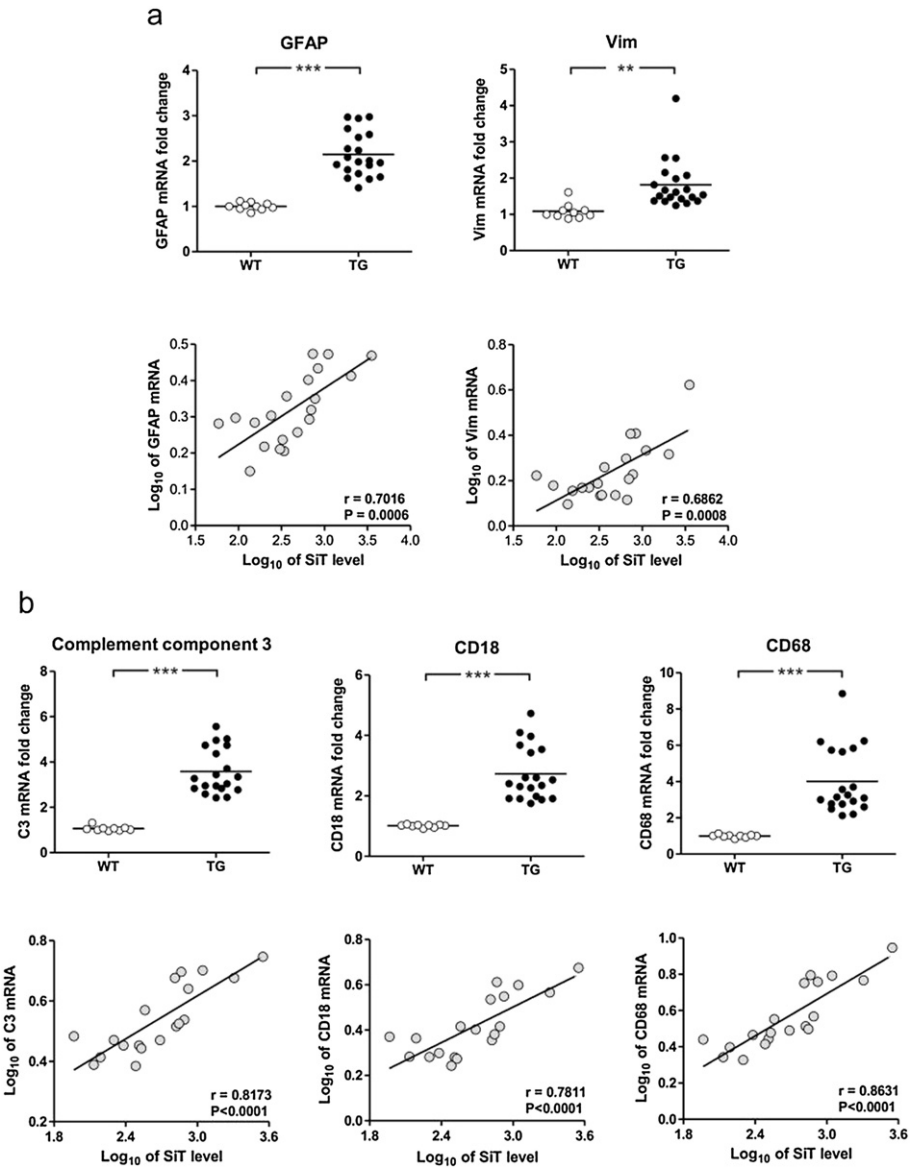

Fig. 7. Increased astrocyte and microglia markers correlate with sarkosyl insoluble tau. Transcriptomic analysis revealed significant upregulation of mRNA expression levels of astrocyte markers (a) GFAP (2.1-fold, ***P < 0.001) and Vimentin (1.7-fold, **P = 0.0026) and up-regulation of markers of microglia (b) C3 (3.4-fold, ***P < 0.0001), CD18 (2.6-fold, ***P < 0.0001) and CD68 (3.9-fold, ***P < 0.0001). Both astrocyte markers (GFAP, Vim) and all three microglial markers (C3, CD68, CD18) significantly positively correlated with amount of sarkosyl insoluble tau (n = 20).

animals expressing double truncated tau [31]. The amount of NFTs and the load of sarkosyl insoluble tau proteins are related to the stage of the disease and variability in this parameter allows for performing correlation studies using the animals of same age. It was shown, that expression of Hsp27, one of the small stress-inducible/associated proteins correlates with the severity of AD-specific morphological changes, and also with the duration of dementia [44,46]. Consistent with these observations, we detected a strong induction of Hsp27 at both mRNA and protein levels in our rat model. Developmental increase in the level of Hsp27 was demonstrated recently using rTg4510 transgenic mouse model of tauopathy, however this increase between adolescence and adulthood was evident also in control mice, and the tau dependent acceleration of Hsp27 was very modest [15]. In our rat model we observed a slight age-associated increase in the amount of Hsp27 between 2 and 4 months of age. However, in contrast to the P301L mice [15], the level of Hsp27 in double truncated transgenic rats was clearly tau-dependent and was about 3-fold higher than in age matched non-transgenic controls, at both the transcriptional and translational levels.

In 6 month old transgenic rats, the loads of insoluble tau protein correlated strongly with the levels of Hsp27. This relationship corresponds to and extends the phenomenon observed in experimental animals and in human diseased brain tissue [8,15,44]. Here we demonstrated for the first time that the amount of Hsp27 increased proportionally to the load of insoluble tau accumulated in the brain. Our findings are in contrast to data of others [30,45] who showed previously a direct interaction of Hsp27 with phosphorylated tau from AD. These authors [30,45] worked with human material and did not show cell type specificity of Hsp27 expression in the brain. In our experiments misfolded tau does not come in contact with Hsp27, yet strongly induces its expression in adjacent astrocytes.

We also found strong correlations of insoluble tau with markers typical for astrocytes and microglia, such as GFAP, Vimentin and complement components C3, CD18, CD68, respectively. We have demonstrated previously that C4, CD11b/CD18, CD68 are present on microglial cells [50], therefore, here we have focused on analyzing the presence of Hsp27 in astrocytes and degenerating neurons. We found that expression of Hsp27 was located almost exclusively in

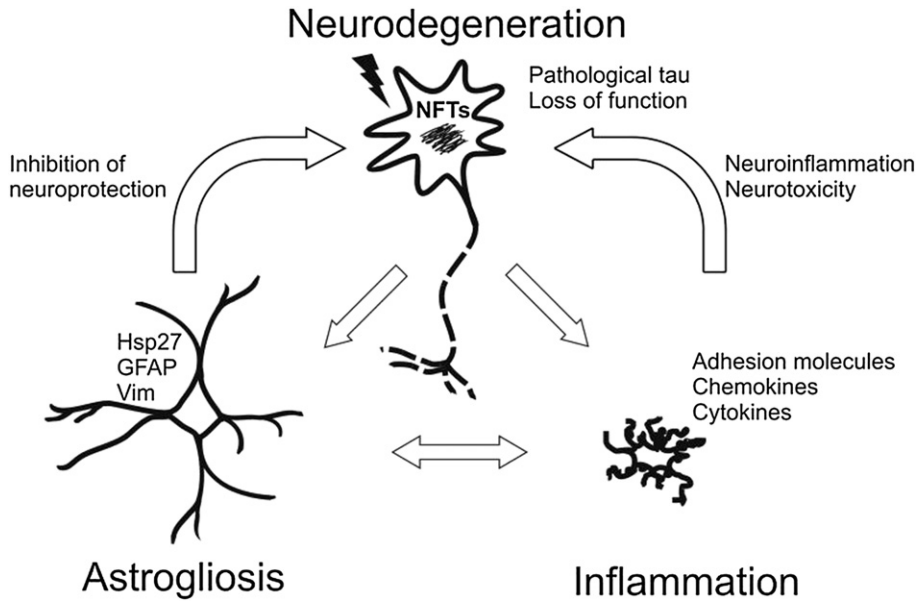

Fig. 8. Proposed interplay and concerted actions between neurons, astrocytes and microglia, induced by expression of pathologically truncated tau protein. Posttranslationally modified tau protein induces intraneuronal accumulation of high molecular weight tau oligomers and insoluble aggregates. This phenomenon is accompanied with deterioration of neuronal function and axonal damage. During this process microglial cells are activated and neuroinflammatory cascades are involved. Subsequent or simultaneous activation of astrocytes may lead to neuroprotection via production of beneficial growth factors and/or through activation of chaperone machinery which may result in increased cell division of neuroprotective astrocytes. However, over time the protective phase of neuroinflammation turns to chronic inflammatory process and the neuroprotective function of astrocytes is inhibited and followed by accelerated neurodegeneration.

astrocytes. Although activated Hsp27 positive astrocytes were located in close proximity of degenerating neurons, we found very rare co-localization of Hsp27 with specific markers of degenerated neurons. It was published that Hsp27 is expressed in astrocytes and oligodendroglia in tauopathies with extensive glial pathology [13,22,23,33], however in our model there is no expression of pathological tau in astrocytes and other glial cells. The pathological tau in the rat transgenic model is expressed exclusively in neurons (driven by Thy1 promoter). Induction of Hsp27 expression in this model is therefore mediated indirectly either via activated microglia expressing immune markers or by stimulation of astrocytes by extracellular tau, which could be released from degenerating neurons (Fig. 8). The transgenic rat model therefore represents a unique experimental tool with cell type specific and topologically dissociated neurofibrillary pathology and expression of Hsp27. Our data point to the specific signaling between affected neurons and activated astrocytes, which could be mediated via e.g. chemokines and/or cytokines and which deserve further investigation (Fig. 8).

Based on our results we suggest that the role of Hsp27 although originally protective may participate on activation of astrocytes paradoxically leading to inhibition of neuroprotective role of astrocytes, which can be observed in chronic neurodegenerative diseases such as tauopathies and AD [55]. This could lead to further impairment of neuronal functions and acceleration of pathogenesis. Further analysis of animals with simultaneous overexpression of pathological forms of tau and down- or up-regulated expression of Hsp27 could shed more light on the neuroprotective/neurotoxic role of Hsp27 and intercellular signaling in the course of neurofibrillary degeneration.

In conclusion, we have demonstrated at both the transcriptional and translational levels that accumulation of sarkosyl insoluble tau strongly correlates with elevated expression of Hsp27, despite their localizations in different cell types. The activated chaperone is localized almost exclusively in activated astrocytes, while NFTs are exclusively produced in neurons. The role of Hsp27 in tauopathies may therefore be mediated indirectly and is different from the canonical role of this chaperone, e.g. folding competency, anti-apoptotic or antioxidant, since Hsp27 does not come into contact with the pathogenic molecule. Hsp27 can be used as a neuropathological marker of activated astrocytes in degenerative diseases such as tauopathies. We suggest that the transgenic rat model employed in this study can be efficiently used for investigation of intercellular signaling between microglia, astrocytes and neurons.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2015.03.003.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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References

[1] J.F. Abisambra, U.K. Jinwal, J.R. Jones, L.J. Blair, J. Koren III, C.A. Dickey, Exploiting the diversity of the heat-shock protein family for primary and secondary tauopathy therapeutics, Curr. Neuropharmacol. 9 (4) (2011) 623–631, http://dx.doi.org/10.2174/157015911798376226.

[2] M.T. Akbar, A.M. Lundberg, K. Liu, S. Vidyadaran, K.E. Wells, H. Dolatshad, S. Wynn, D.J. Wells, D.S. Latchman, J. de Belleroche, The neuroprotective effects of heat shock protein 27 overexpression in transgenic animals against kainate-induced seizures and hippocampal cell death, J. Biol. Chem. 278 (22) (2003) 19956–19965, http://dx.doi.org/10.1074/jbc.M207073200.

[3] R. Arya, M. Mallik, S.C. Lakhotia, Heat shock genes—integrating cell survival and death, J. Biosci. 32 (3) (2007) 595–610.

[4] B. Bandyopadhyay, G. Li, H. Yin, J. Kuret, Tau aggregation and toxicity in a cell culture model of tauopathy, J. Biol. Chem. 282 (22) (2007) 16454–16464, http://dx.doi.org/10.1074/jbc.M700192200.

[5] L.I. Binder, A.L. Guillozet-Bongaarts, F. Garcia-Sierra, R.W. Berry, Tau, tangles, and Alzheimer's disease, Biochim. Biophys. Acta 1739 (2–3) (2005) 216–223, http://dx.doi.org/10.1016/j.bbadis.2004.08.014.

[6] C. Bjorkdahl, M.J. Sjogren, X. Zhou, H. Concha, J. Avila, B. Winblad, J.J. Pei, Small heat shock proteins Hsp27 or alphaB-crystallin and the protein components of neurofibrillary tangles: tau and neurofilaments, J. Neurosci. Res. 86 (6) (2008) 1343–1352, http://dx.doi.org/10.1002/jnr.21589.

[7] H. Braak, K. Del Tredici, The pathological process underlying Alzheimer's disease in individuals under thirty, Acta Neuropathol. 121 (2) (2011) 171–181, http://dx.doi.org/10.1007/s00401-010-0789-4.

[8] H. Braak, K. Del Tredici, Where, when, and in what form does sporadic Alzheimer's disease begin? Curr. Opin. Neurol. 25 (6) (2012) 708–714, http://dx.doi.org/10.1097/WCO.0b013e32835a3432.

[9] L. Buee, A. Delacourte, Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease, Brain Pathol. 9 (4) (1999) 681–693.

[10] R.J. Castellani, G. Perry, The complexities of the pathology–pathogenesis relationship in Alzheimer disease, Biochem. Pharmacol. 88 (4) (2014) 671–676, http://dx.doi.org/10.1016/j.bcp.2014.01.009.

[11] M. Cente, P. Filipcik, S. Mandakova, N. Zilka, G. Krajciova, M. Novak, Expression of a truncated human tau protein induces aqueous-phase free radicals in a rat model of tauopathy: implications for targeted antioxidative therapy, J. Alzheimers Dis. 17 (4) (2009) 913–920, http://dx.doi.org/10.3233/JAD-2009-1107.

[12] N. Csokova, R. Skrabana, H.D. Liebig, A. Mederlyova, P. Kontsek, M. Novak, Rapid purification of truncated tau proteins: model approach to purification of functionally active fragments of disordered proteins, implication for neurodegenerative diseases, Protein Expr. Purif. 35 (2) (2004) 366–372, http://dx.doi.org/10.1016/j.pep.2004.01.012.

[13] D.V. Dabir, J.Q. Trojanowski, C. Richter-Landsberg, V.M. Lee, M.S. Forman, Expression of the small heat-shock protein alphaB-crystallin in tauopathies with glial pathology, Am. J. Pathol. 164 (1) (2004) 155–166.

[14] D. Davila, E.M. Jimenez-Mateos, C.M. Mooney, G. Velasco, D.C. Henshall, J.H. Prehn, Hsp27 binding to the 3′UTR of bim mRNA prevents neuronal death during oxidative stress-induced injury: a novel cytoprotective mechanism, Mol. Biol. Cell 25 (21) (2014) 3413–3423, http://dx.doi.org/10.1091/mbc.E13-08-0495.

[15] C. Dickey, C. Kraft, U. Jinwal, J. Koren, A. Johnson, L. Anderson, L. Lebson, D. Lee, D. Dickson, R. de Silva, L.I. Binder, D. Morgan, J. Lewis, Aging analysis reveals slowed tau turnover and enhanced stress response in a mouse model of tauopathy, Am. J. Pathol. 174 (1) (2009) 228–238, http://dx.doi.org/10.2353/ajpath.2009.080764.

[16] P. Filipcik, N. Zilka, O. Bugos, J. Kucerak, P. Koson, P. Novak, M. Novak, First transgenic rat model developing progressive cortical neurofibrillary tangles, Neurobiol. Aging 33 (7) (2012) 1448–1456, http://dx.doi.org/10.1016/j.neurobiolaging.2010.10.015.

[17] T.B. Franklin, A.M. Krueger-Naug, D.B. Clarke, A.P. Arrigo, R.W. Currie, The role of heat shock proteins Hsp70 and Hsp27 in cellular protection of the central nervous system, Int. J. Hyperth. 21 (5) (2005) 379–392, http://dx.doi.org/10.1080/02656730500069955.

[18] M. Goedert, M.G. Spillantini, Pathogenesis of the tauopathies, J. Mol. Neurosci. 45 (3) (2011) 425–431, http://dx.doi.org/10.1007/s12031-011-9593-4.

[19] I. Grundke-Iqbal, K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, L.I. Binder, Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology, Proc. Natl. Acad. Sci. U. S. A. 83 (13) (1986) 4913–4917.

[20] P.M. Horowitz, K.R. Patterson, A.L. Guillozet-Bongaarts, M.R. Reynolds, C.A. Carroll, S.T. Weintraub, D.A. Bennett, V.L. Cryns, R.W. Berry, L.I. Binder, Early N-terminal changes and caspase-6 cleavage of tau in Alzheimer's disease, J. Neurosci. Off. J. Soc. Neurosci. 24 (36) (2004) 7895–7902, http://dx.doi.org/10.1523/JNEUROSCI.1988-04.2004.

[21] M. Hutton, C.L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraverty, A. Isaacs, A. Grover, J. Hackett, J. Adamson, S. Lincoln, D. Dickson, P. Davies, R.C. Petersen, M. Stevens, E. de Graaff, E. Wauters, J. van Baren, M. Hillebrand, M. Joosse, J.M. Kwon, P. Nowotny, L.K. Che, J. Norton, J.C. Morris, L.A. Reed, J. Trojanowski, H. Basun, L. Lannfelt, M. Neystat, S. Fahn, F. Dark, T. Tannenberg, P.R. Dodd, N. Hayward, J.B. Kwok, P.R. Schofield, A. Andreadis, J. Snowden, D. Craufurd, D. Neary, F. Owen, B.A. Oostra, J. Hardy, A. Goate, J. van Swieten, D. Mann, T. Lynch, P. Heutink, Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17, Nature 393 (6686) (1998) 702–705, http://dx.doi.org/10.1038/31508.

[22] S. Chen, I.R. Brown, Neuronal expression of constitutive heat shock proteins: implications for neurodegenerative diseases, Cell Stress Chaperones 12 (1) (2007) 51–58.

[23] A.M. Chow, D.W. Tang, A. Hanif, I.R. Brown, Localization of heat shock proteins in cerebral cortical cultures following induction by celastrol, Cell Stress Chaperones 19 (6) (2014) 845–851, http://dx.doi.org/10.1007/s12192-014-0508-5.

[24] K. Iqbal, C. Alonso Adel, S. Chen, M.O. Chohan, E. El-Akkad, C.X. Gong, S. Khatoon, B. Li, F. Liu, A. Rahman, H. Tanimukai, I. Grundke-Iqbal, Tau pathology in Alzheimer disease and other tauopathies, Biochim. Biophys. Acta 1739 (2–3) (2005) 198–210, http://dx.doi.org/10.1016/j.bbadis.2004.09.008.

[25] N. Jaya, V. Garcia, E. Vierling, Substrate binding site flexibility of the small heat shock protein molecular chaperones, Proc. Natl. Acad. Sci. U. S. A. 106 (37) (2009) 15604–15609, http://dx.doi.org/10.1073/pnas.0902177106.

[26] I. Khlistunova, J. Biernat, Y. Wang, M. Pickhardt, M. von Bergen, Z. Gazova, E. Mandelkow, E.M. Mandelkow, Inducible expression of Tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but can be reversed by inhibitor drugs, J. Biol. Chem. 281 (2) (2006) 1205–1214, http://dx.doi.org/10.1074/jbc.M507753200.

[27] M. King, F. Nafar, J. Clarke, K. Mearow, The small heat shock protein Hsp27 protects cortical neurons against the toxic effects of beta-amyloid peptide, J. Neurosci. Res. 87 (14) (2009) 3161–3175, http://dx.doi.org/10.1002/jnr.22145.

[28] M. Korenova, N. Zilka, Z. Stozicka, O. Bugos, I. Vanicky, M. Novak, NeuroScale, the battery of behavioral tests with novel scoring system for phenotyping of transgenic rat model of tauopathy, J. Neurosci. Methods 177 (1) (2009) 108–114, http://dx.doi.org/10.1016/j.jneumeth.2008.09.027.

[29] P. Korhonen, T. van Groen, A. Thornell, S. Kyrylenko, M.L. Soininen, J. Ojala, E. Peltomaa, H. Tanila, A. Salminen, E.M. Mandelkow, H. Soininen, Characterization of a novel transgenic rat carrying human tau with mutation P301L, Neurobiol. Aging 32 (12) (2011) 2314–2315, http://dx.doi.org/10.1016/j.neurobiolaging.2009.12.022.

[30] K.S. Kosik, H. Shimura, Phosphorylated tau and the neurodegenerative foldopathies, Biochim. Biophys. Acta 1739 (2–3) (2005) 298–310, http://dx.doi.org/10.1016/j.bbadis.2004.10.011.

[31] P. Koson, N. Zilka, A. Kovac, B. Kovacech, M. Korenova, P. Filipcik, M. Novak, Truncated tau expression levels determine life span of a rat model of tauopathy without causing neuronal loss or correlating with terminal neurofibrillary tangle load, Eur. J. Neurosci. 28 (2) (2008) 239–246, http://dx.doi.org/10.1111/j.1460-9568.2008.06329.x.

[32] B. Kovacech, R. Skrabana, M. Novak, Transition of tau protein from disordered to misordered in Alzheimer's disease, Neurodegener. Dis. 7 (1–3) (2010) 24–27, http://dx.doi.org/10.1159/000283478.

[33] I. Lopez-Gonzalez, M. Carmona, L. Arregui, G.G. Kovacs, I. Ferrer, alphaB-crystallin and HSP27 in glial cells in tauopathies, Neuropathology (2014)http://dx.doi.org/10.1111/neup.12134.

[34] K. Maeda, R. Idehara, A. Hashiguchi, H. Takashima, A family with distal hereditary motor neuropathy and a K141Q mutation of small heat shock protein HSPB1, Intern. Med. 53 (15) (2014) 1655–1658.

[35] P. Majerova, M. Zilkova, Z. Kazmerova, A. Kovac, K. Paholikova, B. Kovacech, N. Zilka, M. Novak, Microglia display modest phagocytic capacity for extracellular tau oligomers, J. Neuroinflammation 11 (1) (2014) 161, http://dx.doi.org/10.1186/s12974-014-0161-z.

[36] P.J. McMillan, B.C. Kraemer, L. Robinson, J.B. Leverenz, M. Raskind, G. Schellenberg, Truncation of tau at E391 promotes early pathologic changes in transgenic mice, J. Neuropathol. Exp. Neurol. 70 (11) (2011) 1006–1019, http://dx.doi.org/10.1097/NEN.0b013e31823557fb.

[37] R. Mena, P.C. Edwards, C.R. Harrington, E.B. Mukaetova-Ladinska, C.M. Wischik, Staging the pathological assembly of truncated tau protein into paired helical filaments in Alzheimer's disease, Acta Neuropathol. 91 (6) (1996) 633–641.

[38] K. Nakamura, A. Greenwood, L. Binder, E.H. Bigio, S. Denial, L. Nicholson, X.Z. Zhou, K.P. Lu, Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease, Cell 149 (1) (2012) 232–244, http://dx.doi.org/10.1016/j.cell.2012.02.016.

[39] M. Novak, R. Jakes, P.C. Edwards, C. Milstein, C.M. Wischik, Difference between the tau protein of Alzheimer paired helical filament core and normal tau revealed by epitope analysis of monoclonal antibodies 423 and 7.51, Proc. Natl. Acad. Sci. U. S. A. 88 (13) (1991) 5837–5841.

[40] M. Novak, J. Kabat, C.M. Wischik, Molecular characterization of the minimal protease resistant tau unit of the Alzheimer's disease paired helical filament, EMBO J. 12 (1) (1993) 365–370.

[41] G. Perry, A. Nunomura, K. Hirai, X. Zhu, M. Perez, J. Avila, R.J. Castellani, C.S. Atwood, G. Aliev, L.M. Sayre, A. Takeda, M.A. Smith, Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radic. Biol. Med. 33 (11) (2002) 1475–1479.

[42] D.E. Read, A.M. Gorman, Heat shock protein 27 in neuronal survival and neurite outgrowth, Biochem. Biophys. Res. Commun. 382 (1) (2009) 6–8, http://dx.doi.org/10.1016/j.bbrc.2009.02.114.

[43] C. Reitz, R. Mayeux, Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers, Biochem. Pharmacol. 88 (4) (2014) 640–651, http://dx.doi.org/10.1016/j.bcp.2013.12.024.

[44] K. Renkawek, G.J. Bosman, W.W. de Jong, Expression of small heat-shock protein hsp 27 in reactive gliosis in Alzheimer disease and other types of dementia, Acta Neuropathol. 87 (5) (1994) 511–519.

[45] H. Shimura, Y. Miura-Shimura, K.S. Kosik, Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival, J. Biol. Chem. 279 (17) (2004) 17957–17962, http://dx.doi.org/10.1074/jbc.M400351200.

[46] H. Shinohara, Y. Inaguma, S. Goto, T. Inagaki, K. Kato, Alpha B crystallin and HSP28 are enhanced in the cerebral cortex of patients with Alzheimer's disease, J. Neurol. Sci. 119 (2) (1993) 203–208.

[47] M.G. Spillantini, M. Goedert, Tau protein pathology in neurodegenerative diseases, Trends Neurosci. 21 (10) (1998) 428–433.

[48] G.J. Stege, K. Renkawek, P.S. Overkamp, P. Verschuure, A.F. van Rijk, A. Reijnen-Aalbers, W.C. Boelens, G.J. Bosman, W.W. de Jong, The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity, Biochem. Biophys. Res. Commun. 262 (1) (1999) 152–156, http://dx.doi.org/10.1006/bbrc.1999.1167.

[49] R.A. Stetler, G. Cao, Y. Gao, F. Zhang, S. Wang, Z. Weng, P. Vosler, L. Zhang, A. Signore, S.H. Graham, J. Chen, Hsp27 protects against ischemic brain injury via attenuation of a novel stress-response cascade upstream of mitochondrial cell death signaling, J. Neurosci. Off. J. Soc. Neurosci. 28 (49) (2008) 13038–13055, http://dx.doi.org/10.1523/JNEUROSCI.4407-08.2008.

[50] Z. Stozicka, N. Zilka, P. Novak, B. Kovacech, O. Bugos, M. Novak, Genetic background modifies neurodegeneration and neuroinflammation driven by misfolded human tau protein in rat model of tauopathy: implication for immunomodulatory approach to Alzheimer's disease, J. Neuroinflammation 7 (2010) 64, http://dx.doi.org/10.1186/1742-2094-7-64.

[51] A. Sydow, A. Van der Jeugd, F. Zheng, T. Ahmed, D. Balschun, O. Petrova, D. Drexler, L. Zhou, G. Rune, E. Mandelkow, R. D'Hooge, C. Alzheimer, E.M. Mandelkow, Reversibility of Tau-related cognitive defects in a regulatable FTD mouse model, J. Mol. Neurosci. 45 (3) (2011) 432–437, http://dx.doi.org/10.1007/s12031-011-9604-5.

[52] T. Togo, N. Sahara, S.H. Yen, N. Cookson, T. Ishizawa, M. Hutton, R. de Silva, A. Lees, D.W. Dickson, Argyrophilic grain disease is a sporadic 4-repeat tauopathy, J. Neuropathol. Exp. Neurol. 61 (6) (2002) 547–556.
[53] M. Tolnay, A. Probst, Frontotemporal lobar degeneration—tau as a pied piper? Neurogenetics 4 (2) (2002) 63–75.
[54] M.E. Toth, V. Szegedi, E. Varga, G. Juhasz, J. Horvath, E. Borbely, B. Csibrany, R. Alfoldi, N. Lenart, B. Penke, M. Santha, Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice, Cell Stress Chaperones 18 (6) (2013) 759–771, http://dx.doi.org/10.1007/s12192-013-0428-9.
[55] A. Verkhratsky, M. Olabarria, H.N. Noristani, C.Y. Yeh, J.J. Rodriguez, Astrocytes in Alzheimer's disease, Neurotherapeutics 7 (4) (2010) 399–412, http://dx.doi.org/10.1016/j.nurt.2010.05.017.
[56] A. Vidyasagar, N.A. Wilson, A. Djamali, Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target, Fibrogenesis Tissue Repair 5 (1) (2012) 7, http://dx.doi.org/10.1186/1755-1536-5-7.
[57] V. Vleminckx, P. Van Damme, K. Goffin, H. Delye, L. Van Den Bosch, W. Robberecht, Upregulation of HSP27 in a transgenic model of ALS, J. Neuropathol. Exp. Neurol. 61 (11) (2002) 968–974.
[58] M.M. Wilhelmus, R.M. de Waal, M.M. Verbeek, Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease, Mol. Neurobiol. 35 (3) (2007) 203–216.
[59] C.M. Wischik, M. Novak, H.C. Thogersen, P.C. Edwards, M.J. Runswick, R. Jakes, J.E. Walker, C. Milstein, M. Roth, A. Klug, Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease, Proc. Natl. Acad. Sci. U. S. A. 85 (12) (1988) 4506–4510.
[60] N. Zilka, P. Filipcik, P. Koson, L. Fialova, R. Skrabana, M. Zilkova, G. Rolkova, E. Kontsekova, M. Novak, Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration in vivo, FEBS Lett. 580 (15) (2006) 3582–3588, http://dx.doi.org/10.1016/j.febslet.2006.05.029.
[61] M. Zilkova, N. Zilka, A. Kovac, B. Kovacech, R. Skrabana, M. Skrabanova, M. Novak, Hyperphosphorylated truncated protein tau induces caspase-3 independent apoptosis-like pathway in the Alzheimer's disease cellular model, J. Alzheimers Dis. 23 (1) (2011) 161–169, http://dx.doi.org/10.3233/JAD-2010-101434.